Spectroscopic behavior of pyrrolanthrone and its derivative in aprotic and protic solvents

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ABSTRACT

Recently synthesized pyrrolanthrone, naphtho[1,2,3-cd]indole-6(2H)-one (PyAn), and its water-soluble derivative, 3(naphtho[1,2,3-cd]indole-6(2H)-one-2-yl) sodium propylsulfonate, are very promising for anticancer therapy due to both the fluorescent and cytotoxic properties. The present study is focused on the spectroscopic analysis of solvent effects in PyAn and its derivative. An increase of the solvent polarity results in the bathochromic shift in emission and absorption spectra that indicates the involvement of ππ*-type transition. The double linear correlation of Stokes shift with bulk solvent polarity functions (in terms of Lippert's, Bakhshiev's and Chamma-Viallet's models) and microscopic solvent polarity parameter $E_T^N(30)$ for aprotic and protic solvents is observed. Both general and specific solvent effects are revealed for the solute-solvent systems. Fluorescence quantum yield, fluorescence lifetime and excited-state dipole moment were defined for PyAn and its derivative in different solvents for the first time. The obtained information is of a great importance for the characterization of intermolecular interactions of drugs with biomolecules for the development of new drug delivery systems.

Keywords: naphtho[1,2,3-cd]indole-6(2n)-one, absorption, fluorescence, quantum yield, solvatochromism, polarity function, solute-solvent interaction, excited-state dipole moment

1. INTRODUCTION

Naphtho[1,2,3-cd]indol-6(2H)-one (PyAn) and its derivatives are very promising as luminophores [1], laser dyes [2], and optical sensitizers [3]. Structural similarity of PyAn with known anticancer drugs (anthra[1,9-cd] pyrazole-6(2H)-one) [4] stimulates an interest in the synthesis and study of its novel derivatives. Recently, novel PyAn synthetic procedure has been reported [5], which makes it possible to synthesize a wide range of its derivatives.

In order to promote effective anticancer drugs, information about the specific features of intermolecular interaction with solvents and potential drug delivery systems are very relevant. Such data include the solubility in aqueous and mixed solvents, the opportunity to participate in specific interactions, the information on the change in dipole moments in the excited state. It is known that the difference in ground- and excite-state dipole moments could result in photoinduced binding / removal from proton-containing biological molecules [6] acting as a delivery system. In addition, the information about the high fluorescent properties of molecules makes it possible to use the drugs as a tracer in the processes of transport and accumulation in tissues.

It is commonly recognized, that the nature of solvent influences the spectroscopic behavior of a dissolved chromophore. The intensity, line shape, position of the absorption and emission spectra in solution depend on the type of solute-solvent interactions [7]. In the present work, the effect of different solvents on the absorption and fluorescent properties (position of maxima, fluorescence quantum yield and lifetime) of PyAn and its water-soluble derivative was investigated, the types of electronic transition and solute-solvent interactions by means of the set of solvatochromic models were revealed.

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2. MATERIALS AND METHODS

2.1 Synthesis of water-soluble 3(naphtho[1,2,3-cd]indole-6(2H)-one-2-yl) sodium propylsulfonate

The water-insoluble naphtho[1,2,3-cd]indole-6(2H)-one) (**1**) is synthesized by the new procedure [5]. Its water-soluble derivative 3(naphtho[1,2,3-cd]indole-6(2H)-one-2-yl) sodium propylsulfonate (**2**) is obtained from 2-(3-chloropropyl)naphtho[1,2,3-cd]indole-6(2H)-one, which synthesis is reported in [8]. The synthesis of PyAn derivative is given below.

0.9 g (0.003 mole) of 2-(3-chloropropyl)naphtho[1,2,3-cd]indole-6(2H)-one was boiled with 0.76 g (0.003 mole) of sodium sulfite ($Na_2SO_3 \cdot 7H_2O$) in a solution with 20 ml propanol-2 and 10 ml water for 30 hours. The reaction was monitored by thin-layer chromatography. After a complete reflux of the initial compounds, 200 ml of hot (40-50°C) water was added to the reaction mixture, then the mixture was heated up to 60-70°C. After that a hot filtration was carried out, the filtrate was evaporated in vacuum to a volume of ~ 20 ml. Finally, the formed precipitate was filtered off, washed with 20-25 ml of alcohol, and dried. Yield 0.61 g (67.7%), light brown crystals, mp ≥ 300°C.

^{1}H NMR spectrum, δ, ppm (J, Hz): 2.18 (m, $\underline{CH_2}CH_2CH_2$); 2.50 (t, $CH_2\underline{CH_2}CH_2$); 4.51 (t, $CH_2CH_2\underline{CH_2}$); 7.41 (m, 1H, H4, J 7.5); 7.56 (t, 1H, H8(9), J 7.5); 7.71 (m, 1H, H9(8), J 7.5); 7.98 (d, 1H, H3, J 7.5); 8.04 (d, 1H, H5, J 7.5); 8.06 (d, 1H, H10, J 7.5); 8.30 (d, 1H, H7, J 7.5); 8.47 (s, 1H, H1, J).

The molecular structures of studied compounds are given in Figure 1.

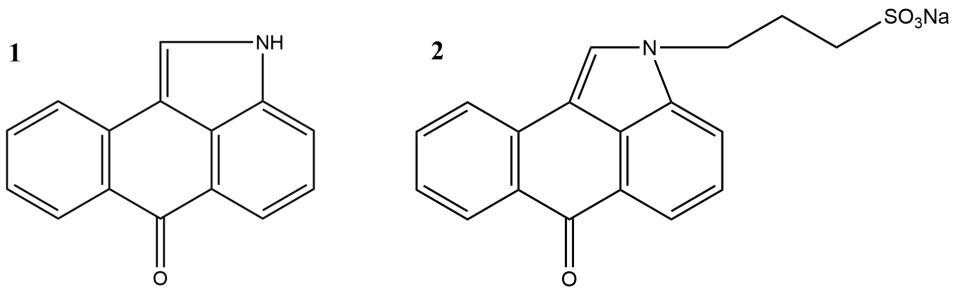

Figure 1. Molecular structure of the PyAn **1** and its water-soluble derivative **2**.

2.2 Steady-state and time-resolved measurements

The absorption and fluorescence spectra of PyAn **1** and its derivative **2** were measured in aprotic (toluene, ethyl acetate, dichloromethane (DCM), N,N-dimethylformamide (DMF) and toluene-DMF mixture) and protic (2-propanol, ethanol, methanol and ethanol-water mixture) solvents. All the used solvents were transparent and nonfluorescent in the studied range of excitation and emission. The absorption spectra were recorded using Lambda 35 spectrophotometer (Perkin Elmer) and fluorescence spectra were recorded using Fluorolog 3-22 spectrofluorometer with additional time-resolved module FluoroHub B (Horiba Jobin Yvon). All the measurements were carried out at room temperature keeping compound concentration very low to avoid the reabsorption.

The quantum yield (Φ) was defined by reference method [7]:

$$\Phi = \Phi_R \frac{I A_R n^2}{I_R A n_R^2}. \quad (1)$$

where I and A are the integrated intensity and absorbance at excitation wavelength, n is the solvent refractive index. The subscript R corresponds to the reference fluorophore of known quantum yield (rhodamine 6G in ethanol, 88% [9] and flavin mononucleotide in water, 26% [10]). The calculations and fitting procedure were performed in Origin 7.0 program.

The fluorescence decays were measured in the emission band maximum upon excitation by laser diode NanoLED (λ_{max} = 373 nm) with pulse duration of < 1.2 ns. The deconvolution procedure and the analysis of the fluorescence decay were performed using the DAS6 program (Horiba). The decays for all samples were fitted by a sum of two or three exponents,

and then the average lifetime was calculated. Standard quartz cells with cross sections of 10 × 10 mm were used to investigate solutions for L-geometry of excitation.

2.3 Solvatochromic shift models

The method of solvatochromic shifts is based on Onsager reaction field theory [11]. It reveals the relationship between the position of the electronic spectra and the fundamental characteristics of the bulk solvent and the studied molecule. Commonly used, Lippert (2) [12], Bakshiev (3) [13] and Chamma-Viallet (4) [14] models correlate the wavenumbers of maxima of absorption ($\bar{\nu}_a$) and fluorescence ($\bar{\nu}_f$) spectra with dielectric constant (ε) and solvent refractive index (n)

$$(\bar{\nu}_a - \bar{\nu}_f) = mF(\varepsilon, n) + const\,, \tag{2}$$

$$(\bar{\nu}_a - \bar{\nu}_f) = m_1 F_1(\varepsilon, n) + const\,, \tag{3}$$

$$(\bar{\nu}_a + \bar{\nu}_f)/2 = m_2 F_2(\varepsilon, n) + const\,. \tag{4}$$

The expressions for polarity functions of Lippert $F(\varepsilon, n)$, Bakshiev $F_1(\varepsilon, n)$ and Chamma-Viallet $F_2(\varepsilon, n)$ are given by

$$F(\varepsilon, n) = \frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1}, \tag{5}$$

$$F_1(\varepsilon, n) = \left(\frac{\varepsilon - 1}{\varepsilon + 2} - \frac{n^2 - 1}{n^2 + 2}\right)\frac{2n^2 + 1}{n^2 + 2}, \tag{6}$$

$$F_2(\varepsilon, n) = \frac{2n^2 + 1}{2(n^2 + 2)}\left(\frac{\varepsilon - 1}{\varepsilon + 2} - \frac{n^2 - 1}{n^2 + 2}\right) + \frac{3(n^4 - 1)}{2(n^2 + 2)^2}\,. \tag{7}$$

Plots of $(\bar{\nu}_a - \bar{\nu}_f)$ versus solvent polarity functions $F(\varepsilon, n)$, $F_1(\varepsilon, n)$ and $(\bar{\nu}_a + \bar{\nu}_f)/2$ versus $F_2(\varepsilon, n)$ for different solvents yield the slopes m, m_1 and m_2, respectively, and are given below:

$$m = m_1 = 2(\mu_e - \mu_g)^2 / hca^3\,, \tag{8}$$

$$m_2 = -2(\mu_e^2 - \mu_g^2) / hca^3\,. \tag{9}$$

Here h is the Planck's constant, c is the velocity of light, a is Onsager cavity radius of the solute molecule, which is commonly taken to be equal to 0.8 of the radius of the molecule [15], μ_e and μ_g are the ground- and excited-state permanent dipole moments.

Assuming that the symmetry of the investigated solute molecule remains unchanged upon electronic transition and the ground- and excited-state dipole moments are parallel, based on Eqs. (6) and (7), one can obtain:

$$\mu_g = \left(hca^3/2m_1\right)^{1/2}(m_2 - m_1)/2\,, \tag{10}$$

$$\mu_e = \left(hca^3/2m_1\right)^{1/2}(m_2 + m_1)/2\,. \tag{11}$$

The linear correlation between Stokes shift and solvent polarity function in these models are assumed to be evidence of general solvent effects dominance among solute-solvent interactions. [7, 15].

2.4 Empirical normalized parameter $E_T^N(30)$ of solvent polarity

The empirical normalized microscopic parameter of polarity $E_T^N(30)$ proposed by Dimroth and Reichardt [16] is commonly used for description of the contribution of general and specific solvent effect. The $E_T^N(30)$ values of different binary solvent mixtures can be calculated using empirical one-parameter (f_2/f_1) equation proposed by [17]

$$E_T^N(30) = \frac{E_{T1}^N(30) + x_2\left[(f_2/f_1)E_{T2}^N(30) - E_{T1}^N(30)\right]}{1 + x_2\left[(f_2/f_1) - 1\right]}, \quad (12)$$

where $E_T^N(30)$ is the normalized empirical parameter of solvent polarity observed for the mixed solvent, x_2 is the mole fraction of less polar solvent, $E_{T1}^N(30)$ and $E_{T2}^N(30)$ are the normalized empirical parameters of solvent polarity for pure solvents. The excited-state dipole moment can be obtained by the slope m_3 in the correlation of the spectral shift with $E_T^N(30)$ [18]:

$$(\bar{\nu}_a - \bar{\nu}_f) = m_3 E_T^N(30) + const, \quad (13)$$

$$m_3 = 11307.6\,(\Delta\mu/\Delta\mu_b)^2 (a_b/a)^3, \quad (14)$$

where $\Delta\mu_b$ (9 D) and a_b (6.2 Å) are the dipole moment change on excitation and the Onsager cavity radius of the pyridinium-N-phenolate betaine dye, respectively, and $\Delta\mu$ and a are the corresponding values for the solute molecule of interest. Hence, Eq. (14) can be rearranged as

$$\Delta\mu = \mu_e - \mu_g = \left(\frac{81 m_3}{11307.6(6.2/a)^3}\right)^{1/2}. \quad (15)$$

The analysis of the relation in Eq. (13) could identify the occurrence of a hydrogen bond in the solute-solvent system.

3. RESULTS AND DISCUSSION

3.1 Fluorescence spectra and lifetimes

We studied the absorption and fluorescence spectra of PyAn **1** and its derivative **2** in aprotic and protic solvents. The spectral properties of PyAn **1** and its derivative **2** are highly sensitive to the nature of the solvent. As shown in Figure 2, the position and shape of the absorption and emission bands are dependent on solvent type. The absorption spectra of PyAn **1** demonstrates the resolved vibronic structure in aprotic solvents that disappears in more polar solvents.

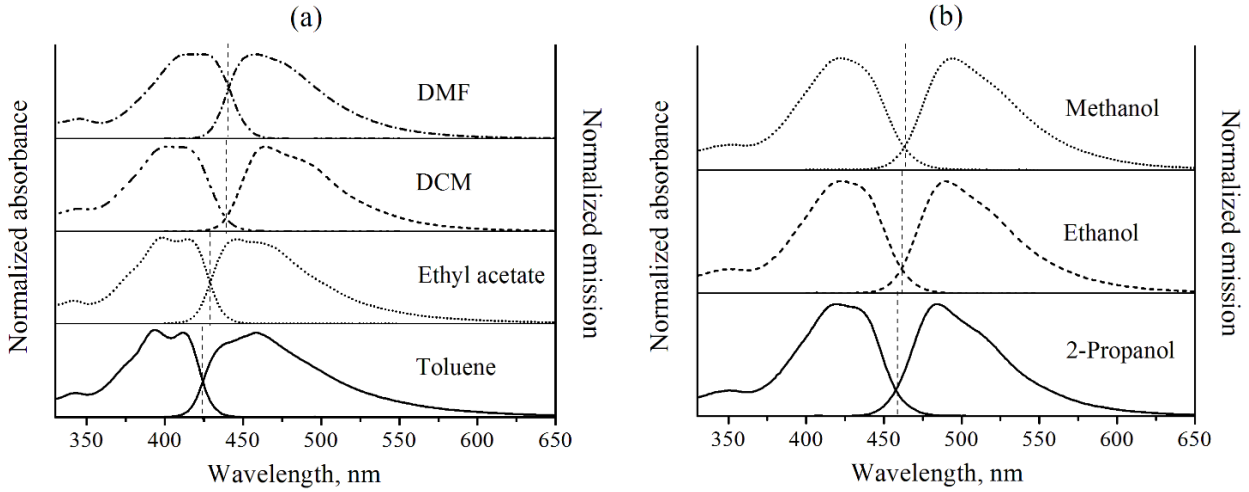

Figure 2. Absorption and fluorescence spectra of PyAn **1** in aprotic (a) and protic (b) solvents.

Bathochromic shifts in fluorescence and absorption spectra were observed with the increase of the solvent polarity. The observed phenomenon can be explained in terms of ππ*-type electronic transition. The shifts in emission spectra in PyAn

1 (~16 nm in aprotic solvent, ~19 nm in protic solvent) are larger than that in absorption spectra (~13 nm in aprotic solvent, ~14 nm in protic solvent). Then, the excited state of PyAn **1** is more sensitive to solvent polarity in comparison to the ground state. The similar spectroscopic behavior was observed for water-soluble derivative **2**, which absorption and emission spectra in water and ethanol are generally red shifted (~12 nm) with respect to the spectra of PyAn **1** (Figure).

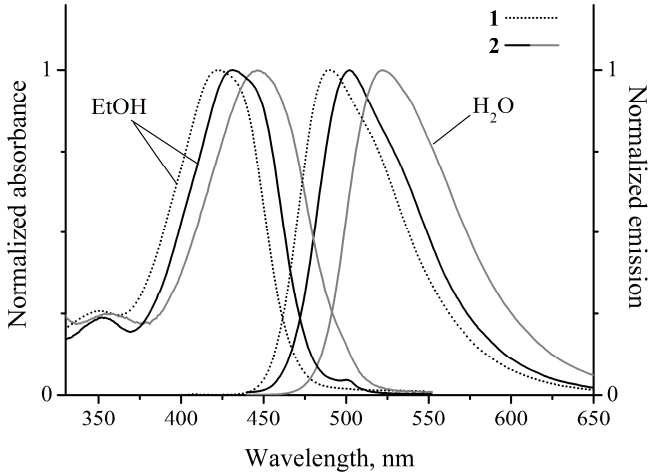

Figure 3. Absorption and fluorescence spectra of PyAn **1** in ethanol (dotted) and its derivative **2** in ethanol (black solid), in water (grey solid).

Figure shows the measured fluorescence quantum yields and lifetimes of the PyAn **1** and its derivative **2** in solvents in wide range of dielectric constants. The type of the solvents significantly influences on fluorescent properties of studied compounds. For example, fluorescence yield and lifetime of PyAn increase from 5% and 1 ns in toluene to ~90% and ~13 ns in alcohols. The fluorescent properties of PyAn derivative **2** in alcohols are similar, but fluorescence yield and lifetime in aprotic solvents exceed the parameters for PyAn **1** (51% and 5.4 ns in toluene). The quantum yield of PyAn derivative **2** in water solution does not exceed 70%.

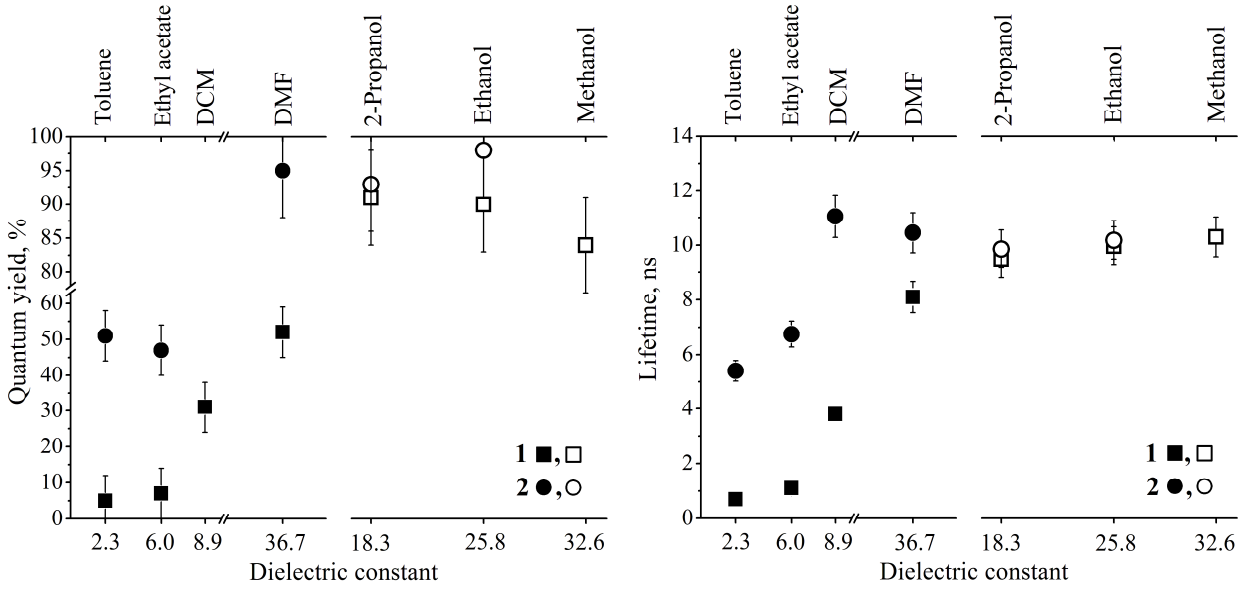

Figure 4. Fluorescence quantum yield and lifetime of PyAn **1** (square) and its derivative **2** (circle) in aprotic (filled) and protic (empty) solvents.

3.2 Solvatochromic effects

In order to get a detailed information about solvatochromic behaviour of PyAn **1** and its derivative **2**, spectroscopic properties were correlated with the different solvent polarity functions. The spectral shifts $(\bar{\nu}_a - \bar{\nu}_f)$ and $(\bar{\nu}_a + \bar{\nu}_f)/2$ of both compounds versus bulk solvent polarity functions $F(\varepsilon,n)$, $F_1(\varepsilon,n)$, $F_2(\varepsilon,n)$ and microscopic solvent polarity parameter E_T^N was plotted. The spectral shifts in aprotic and protic solvents were fitted by two linear functions. Figure shows plots for PyAn **1**. The corresponding values of slopes and correlation coefficients for both compounds are collected in Table 1.

In case of protic solvents, the correlation coefficients exceed the value of 0.90. The slopes of plots for protic solvents are larger than that observed for aprotic solvents. The difference in slopes of the lines for protic and aprotic solvents indicates the contribution of the specific solute-solvent interactions (for instance, hydrogen bond) in addition to the general ones.

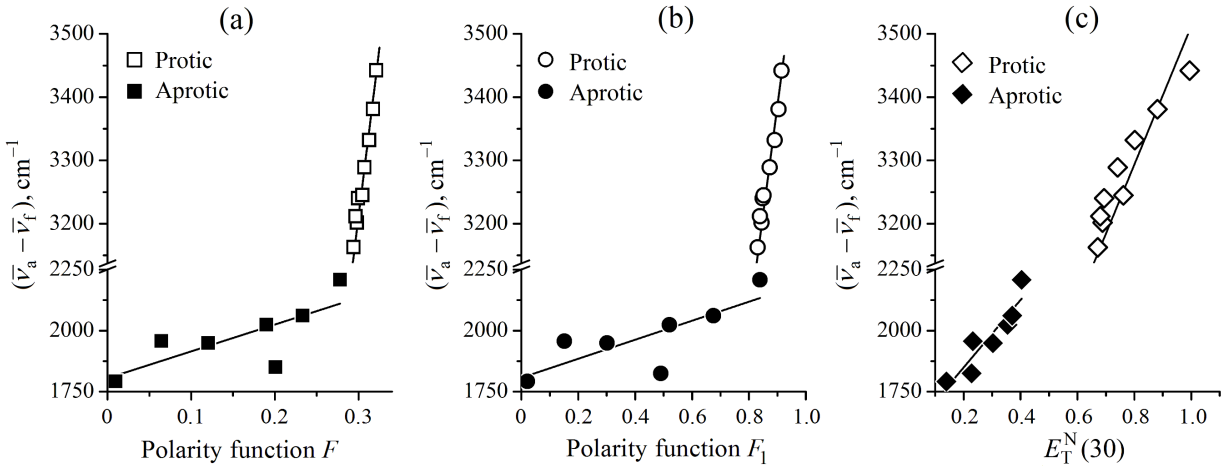

Figure 5. The dependence of Stokes shift on Lippert (a), Bakshiev (b) polarity function and $E_T^N(30)$ (c) for PyAn **1** in aprotic (filled) and protic (empty) solvents.

Table 1. Statistical treatment of the correlations of solvent spectral shifts of PyAn **1** and its water-soluble derivative **2**.

		Lippert		Bakhshiev		Chamma-Viallet		Reichardt	
	Compound	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Protic	Slope m_i, 10^3 cm^{-1}	10.66	9.35	3.41	3.08	-15.69	-17.75	1.09	0.91
	Correlation coefficient	0.99	0.95	0.98	0.98	0.97	0.88	0.95	0.87
Aprotic	Slope m_i, 10^3 cm^{-1}	1.10	0.79	0.39	0.26	-2.88	-1.15	1.38	0.83
	Correlation coefficient	0.76	0.93	0.79	0.94	0.83	0.98	0.92	0.85

The values of $\Delta\mu$, ground-state dipole moment μ_g, excited-state dipole moment μ_e were calculated from the slopes of respective plots (Table 2). These parameters strongly depend on Onsager cavity radius (Eqs. (8)–(10), (14)). All of the used models revealed that the excited-state dipole moment values are higher than those of ground-state ($\mu_e > \mu_g$). The values of μ_g, μ_e and $\Delta\mu$ of compounds **1** and **2** in aprotic and protic solvents are represented in Table 2.

From Table 2 it can be seen that the values of $\Delta\mu$ calculated by the set of solvatochromic shift models differ from each other. The variety of results could be explained by different assumptions upon the derivation of equations (8)–(10), (13). For instance, authors [19] investigating fluorescein argue that Lippert's equation is more sensitive to the specific solute-solvent interactions while Bakshiev's equation is more sensitive to the general solvent effects. In general, the ground-state dipole moment of compound **1** turned out to be lower than that of the compound **2** in protic solvents, the inverse ratio is observed for aprotic solvents. Further progress in the study could be connected with the quantum mechanical computations of intramolecular charge distribution.

Table 2. Estimation of the changes in dipole moments, ground-state and singlet excited-state dipole moment (D).

Compound	Onsager radius, Å	Solvent	$\Delta\mu$			μ_g	μ_e
			Eq. (2), (8)	Eq. (3), (8)	Eq. (15)	Eq. (10)	Eq. (11)
1	3.73	Protic	7.37	4.17	1.70	7.50	11.67
		Aprotic	2.36	1.41	2.15	4.51	5.92
2	4.23	Protic	8.34	4.78	2.07	11.41	16.20
		Aprotic	2.01	1.15	1.28	1.98	3.13

4. CONCLUSIONS

In the work, the solvent effects on spectroscopic behavior of novel promising compounds: pyrrolanthrone and its water-soluble derivative were investigated. The fluorescence quantum yield and lifetime of the PyAn **1** are very sensitive to the nature of the solvents: fluorescence yield (lifetime) varies from 5% (0.7 ns) in toluene to almost 90% (~13 ns) in alcohols. Water-soluble PyAn derivative **2** also demonstrates high fluorescent efficiency: yield (lifetime) varies from 51% (5.4 ns) in toluene to almost 95% (~13 ns) in alcohols while its quantum yield in water does not exceed 70%. The solvent polarity results in bathochromic shift of the absorption and emission spectra due to $\pi\pi^*$-type of electronic transition. Using Lippert's, Bakhshiev's and Chamma-Viallet's solvatochromic shift models as well as the dependence of molecular-microscopic solvent polarity parameter on the spectral shift, the dipole moments of the molecules were estimated. The excited-state dipole moments are 1.70–8.34 D in protic solvents and 1.15–2.36 D in aprotic solvents. These dipole moments are larger than that in the ground state. The observed double linear correlation between Stokes shift and solvent polarity function is commonly treated as both general and specific solvent effects. The obtained results will be useful for the design of anticancer drug delivery systems that do not require external fluorescent labels.

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